Psychological states and physical fates

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Chapter 5

Effects of urinary cortisol levels and resting heart rate on the risk for fatal and nonfatal cardiovascular events

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Submitted

ABSTRACT

Context

Higher cortisol levels are associated with cardiovascular mortality in the elderly. It is unclear whether this association also exists in a general population of younger adults and for non-fatal cardiovascular events. Likewise, resting heart rate is associated with cardiovascular mortality, but fewer studies have also considered non-fatal events.

Objective

The goal of this study was to investigate whether twenty-four-hour urinary cortisol (24-h UFC) levels and resting heart rate (RHR) predict major adverse fatal and non-fatal cardiovascular events (MACE) in the general population.

Design and Setting

We used data from a subcohort of the PREVEND study, a prospective general population based cohort study with a follow-up of 6.4 years for 24-h UFC and 10.6 years for RHR.

Participants

Participants were 3432 adults (mean age 49 years, range 28-75).

Main Outcome Measure

24-h UFC was collected and measured by liquid chromatography—tandem mass spectrometry. RHR was measured at baseline in a supine position for 10 minutes with an automatic device (Dinamap XL Model 9300). Information about cardiovascular events was obtained from the Dutch national registry of hospital discharge diagnoses. Information about mortality was obtained from the municipal register.

Results

After excluding people on corticosteroid medication, a population of 2792 people remained, in which 121 MACE took place. 24-h UFC did not significantly increase the hazard of MACE (hazard ratio=0.999, 95% confidence interval=0.993-1.006, p=.814). After exclusion of people on antihypertensive medication a population of 2823 people remained, in which 156 MACE occurred. RHR increased the risk for MACE with 17% per 10 extra heart beats per minute (hazard ratio=1.016, 95% confidence interval=1.001-1.031, p=.036) after adjustment for conventional risk factors.

Conclusions

We did not find evidence that 24-h UFC increases the risk of MACE. RHR, on the contrary is a potentially modifiable risk factor for MACE in the general population.

INTRODUCTION

Psychosocial stress is a well-known risk factor for cardiovascular disease (CVD)[1]. Hyperactivation of the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic nervous system (SNS), leading to increased cortisol levels and resting heart rate are postulated to be amongst the mechanisms behind this association[2].

This is plausible as cortisol has a direct effect on various risk factors for CVD. Increased levels of cortisol can affect blood pressure[3], BMI[4], waist circumference[4], fasting glucose levels[4], and HDL levels[4]. Moreover, glucocorticoids may also adversely influence remodeling after myocardial infarction via inhibition of angiogenesis[5], and induction of fibrosis via activation of the mineralocorticoid receptor[6]. An elevated resting heart rate (RHR) in turn might influence cardiovascular outcome by increasing cardiac oxygen demand[7], decreasing coronary blood flow by decreasing the duration of the diastole[8], lowering endothelial shear stress[9], and increasing the risk of plague rupture[10].

Two recent prospective studies showed that elevated levels of cortisol predict cardiovascular death amongst elderly people with[11] and without preexisting CVD[11,12]. To our knowledge, studies in a younger population investigating whether physiological levels of endogenous cortisol are associated with increased incidence in cardiovascular events are lacking. Moreover, the studies in elderly people did not investigate the relationship of cortisol levels with non-fatal cardiovascular events. Regarding the effects of RHR, in populations without known CVD, RHR was found to be a risk factor for both cardiovascular death[13–15] and morbidity[14,15], although some studies did not find any relationship with non-fatal cardiovascular events[16–18].

In the current study we assessed for the first time in a general population cohort whether higher levels of cortisol are an independent risk factor for major adverse fatal and non-fatal cardiovascular events (MACE). Moreover, we intended to replicate the results of previous studies with regard to higher RHR and the risk for MACE.

MATERIALS AND METHODS

Study population

We used data from a subcohort of the Prevention of REnal and Vascular ENd stage Disease (PREVEND) study. PREVEND is population cohort study originally designed to investigate microalbuminuria as a risk factor for renal and CVD. The recruitment of participants for PREVEND has been extensively described elsewhere[19]. In brief, 8,592 subjects completed the baseline screening survey in 1997-1998 (T1), rendering the PREVEND study cohort. Subjects with insulin dependent diabetes mellitus and pregnant women were excluded from the study population. The PREVEND study is enriched for albuminuria which is a risk factor for developing renal disease. To obtain a representative random sample of the Groningen general population for the current study, we included all subjects with a urinary albumin concentration (UAC) <10 mg/L that completed the first screening (N=2592) and added a random subset (n=840) from the “overrepresented” subjects with an UAC >10mg/L

proportional to the degree of overrepresentation. This resulted in a group of 3432 subjects with a population representative ratio of albuminuria negative and positive subjects forming the basis for the current study. The average age was 49 years, minimum and maximum were 28 – 75 years. Follow-up measurements took place between January 2002 and November 2003 (T2). Average time between T1 and T2 was 4.1 years. The study was approved by the local Medical Ethical Committee for human research of the University Medical Center Groningen (UMCG). All participants were aged 18 or older and provided written informed consent for participation in this study.

24-hour urinary free cortisol

24-h urinary free cortisol (24-h UFC) was measured at T2. Participants were asked to collect urine samples in a polypropylene container on two consecutive days prior to the visit to the outpatient clinic. They were carefully instructed to urinate into the container during the 24-h collection period and refrigerate the sample until delivery to the laboratory. 24-h urine collection was available on at least one day for 2761 people and at both days for 2710 people. Urinary creatinine was measured to assess completeness of the 24-h urine collection. We used the following formula to assess completeness: incomplete urine=<0.7 of [mmol urinary creatinine x 113]/[21 x kilograms of body weight][20], as this has been proven to be the most sensitive method to detect incompleteness[21]. Only samples which were complete according to the above formula were used for the current study. Urinary free cortisol (UFC) was measured by liquid chromatography—tandem mass spectrometry (LC—MS/MS) analysis. The lower detection limit of the assay was 0.3 nmol / l. At low, middle, and high concentrations, intra-assay variation ranged from 1.3- 2.4% while inter-assay variation ranged from 3.8-7.8%. 24-h UFC was calculated by multiplying urinary volume with cortisol concentration and is expressed in nmol per 24-h. We used the mean of the two samples on two consecutive days to reflect HPA axis function. In the case when values of only one day were available we used this value instead of the mean.

Resting heart rate

RHR and blood pressure Blood pressure was measured, in supine position, every minute for 10 minutes, with an automatic device (Dinamap XL Model 9300, Johnson–Johnson Medical, Tampa, FL, USA).

Follow-up and Outcomes

Information about cardiovascular events was obtained from the Dutch national registry of hospital discharge diagnoses (PRISMANT). Information about mortality was obtained from the municipal register. Information on the cause of death was acquired by linking the number of death certificates to the primary cause of death as coded by the Dutch Central Bureau of Statistics. The outcome of interest was a combined end-point of fatal and non-fatal major adverse cardiovascular events (MACE). MACE was defined as acute myocardial infarction (ICD-code 410), acute and subacute ischemic heart disease (411), occlusion or stenosis of the precerebral (433) or cerebral arteries (434) and the following procedures: coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, and other vascular interventions namely percutaneous transluminal angioplasty or bypass

grafting of aorta and peripheral vessels. Mortality from any other cause was censored.

Medication use

Medication (antihypertensive, lipid-lowering, antidiabetic) was self-reported and substantiated with information of drug-use from the IADB.nl, which contains dispensing information from 55 community pharmacies in the Northern part of the Netherlands, covering on average 500 000 persons annually (www.IADB.nl) and almost the entire population of PREVEND study participants[22]. The database's pharmacy information includes, among others, name of the drug, anatomic–therapeutic–chemical (ATC) classification and date of prescription. Medication records of patients are virtually complete because of high patient pharmacy commitment in the Netherlands[23] We extracted information on drug prescriptions from 100 days prior until 100 days after the date of the visit to our research facilities.

Covariates

Each survey in the PREVEND study consisted of one to two visits to an outpatient unit. Participants completed a questionnaire on demographics, CVD history, lifestyle and medication use before the visit. Height and weight were measured and a fasting blood sample was drawn. Body mass index was calculated as the ratio between weight and height squared. Smoking status was assessed by self-report. Participants were considered smokers if they had smoked in the previous year and previous smokers if they had quit smoking more than one year ago. Hypertension was defined as systolic blood pressure >= 140 mm Hg or diastolic blood pressure >= 90 mm Hg following the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure criteria, or use of antihypertensive medication. Hyperlipidemia was defined as cholesterol level > 6.5 mmol/L when a history of hyperlipidemia was absent, or use of lipid- lowering drugs. Diabetes was defined as fasting glucose level 7.0 mmol/L, nonfasting glucose level 11.1 mmol/L, or use of antidiabetic medication. Prior history of CVD at inclusion of the study was defined as self-report of cerebrovascular accident, coronary heart disease, or peripheral vascular disease requiring surgery.

Statistical analysis

For statistical analysis, 24-h UFC and RHR were analyzed as a continuous measure, and divided into quintiles to assess if the association was linear. We used Cox proportional hazards regression to investigate the associations between 24-h UFC or RHR (in separate models), and the outcome variable MACE. Model 1 was adjusted only for sex and age. Model 2 was additionally adjusted for smoking status, hypertension, hyperlipidemia, diabetes mellitus, and history of CVD. Model 3 was the same as model 2, but with additional adjustment for creatinine clearance and BMI. The lowest category (e.g. the 1st quintile) was always used as a reference category. For sex, males were used as the reference category. The use of corticosteroids leads to negative feedback on the HPA axis and thus to lower endogenous levels of 24-h UFC. Thus, for the analysis of 24-h UFC, we excluded participants using inhalation, local, gastrointestinal, or systemic glucocorticosteroids (15%). For the analyses on the effects of RHR, we excluded people on antihypertensive medication (14%) as this might

either directly (beta-blocker, calcium channel blocker) or indirectly influence the values of RHR. The proportional hazards assumption was checked by graphically assessing the log-log minus function of all predictor variables. The assumption was met for all predictor variables in all analyses. Unlike RHR, 24-h UFC was not measured at baseline, but only at the second assessment wave (T2). As any longitudinal study, PREVEND suffered from some loss to follow-up. We therefore used multiple imputation techniques to correct for attrition bias (supplement 1). The conclusions drawn from the Cox regression models on the original data and the imputed data sets were in accordance with each other. The estimates below are pooled estimates of the imputed data. Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 20.0. and Stata Statistical Software: Release 12. StataCorp. A two-sided P value of ≤0.05 was considered statistically significant.

RESULTS

Population characteristics at T1 and T2

Descriptive statistics of the characteristics of our study population at T1 and T2 are provided in table 1. There were slightly more women than men at T1 and T2 (56%). The average age at T1 was 49 years with a minimum of 28 and a maximum of 75 years. The median follow-up time from T1 was 10.6 years and the median follow-up time from T2 was 6.4 years. From T1 until follow-up in January 2009, a total of 263 MACE took place. From T2 until follow-up in January 2009 a total of 161 MACE took place. During the PREVEND study, 588 T1 participants (17%) did not show up at the follow-up visit (T2).

The prediction of MACE by 24-hour urinary free cortisol

After excluding people on corticosteroid medication, a sample of 2792 people remained, in which 121 MACE took place. Table 2 shows the results of the three Cox proportional hazard models assessing the association between quintiles of 24-h UFC and MACE. 24-h UFC did not significantly influence the hazard of MACE. The traditional risk factors for MACE: sex, age, smoking status, hypertension, hyperlipidemia, and previous history of CVD were all highly significant. To exclude the possibility that our negative findings could be explained by the use of artificial cut-off scores, we also ran two models adjusted only for sex and age with the continuous measure of 24-h UFC as a predictor. MACE was not significantly predicted by the continuous measure of 24-h UFC (p=.814). To investigate whether 24-h UFC levels might be only a significant predictor for people of a certain age, we also investigated a model with a multiplicative interaction term between 24-h UFC and age. The interaction term between 24-h UFC and age (p=.791) was not statistically significant. Likewise, an interaction term between sex and 24-h UFC (p=.090) was not significant. We excluded the possibility that our null findings could be explained by mixing a population with and without a history of CVD by rerunning the analyses excluding persons with a history of CVD. After excluding those with a history of CVD, a population of 2687 people remained in which 97 MACE took place. 24-h UFC was again not a significant predictor of MACE (p=.923).

Table 1. Population characteristics at T1 and T2

Variable	**T1**	**T2**
	N=3432	N=2773*
MACE (N)	263	161
Myocardial infarction	72	40
(Sub)acute ischeamic heart disease	63	39
Occlusion or stenosis of:		
Precerebral arteries	14	8
Cerebral arteries	27	19
CABG	22	14
PTCA	26	14
PTA of peripheral vessels	2	1
Bypass grafting of aorta	10	5
Carotid endarterectomy or carotid stenting	7	6
Death by MACE	20	15
Mean survival time MACE (SE)	3925 (12)	2719 (7)
Age	49 (12)	53 (12)
Sex (female)	56%	56%
Cortisol in nmol/24-h	n.a.	69 (47-96)
Quintiles:		
1st	n.a.	1≤UFC≤43
2nd	n.a.	43<UFC≤59
3rd	n.a.	59<UFC≤79
4th	n.a.	79<UFC≤106
5th	n.a.	106<UFC≤666
Resting heart rate	69 (10)	n.a.
Quintiles:		
1st	42≤RHR≤61	n.a.
2nd	61<RHR≤66	n.a.
3rd	66<RHR≤71	n.a.
4th	71<RHR≤77	n.a.
5th	77<RHR≤115	n.a.
Smoking		
Never smoked	32%	32%
Previous smoker	33%	40%
Current smoker	35%	28%
BMI	26 (4)	27 (4)
Creatinine clearance	101 (26)	102 (25)
Hypertension	27%	28%
Diabetes (yes)	3%	5%
Hyperlipidemia (yes)	23%	22%
History of CVD (yes)	5%	4%
Antihypertensive medication (yes)	14%	18%
Glucocorticoid medication (yes)	n.a.	15%

**Unimputed data after the exclusion of people with events before T2. CVD=cardiovascular disease, BMI=body mass index, MACE= a combined end-point of fatal and non-fatal Major Adverse Cardiovascular Event, N=number of events, SE=standard error of the mean,CABG= coronary artery bypass grafting, PTCA= percutaneous transluminal coronary angioplasty, PTA= percutaneous transluminal angioplasty, UFC=urinary free cortisol, RHR=resting heart rate, n.a. not applicable. Descriptives are given of non-missing values as either percentages or mean with standard deviation between brackets unless indicated otherwise.*

Table 2. Association of MACE with 24-h UFC in Multivariable Cox Regression Models*

	Model 1			Model 2			Model 3		
Variable	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
24-h UFC 2nd	0.741	0.253–2.170	.583	0.820	0.273–2.469	.724	0.823	0.273–2.479	.728
24-h UFC 3rd	0.710	0.272–1.859	.485	0.740	0.275–1.991	.550	0.745	0.276–2.010	.560
24-h UFC 4th	0.844	0.313–2.278	.737	0.916	0.328–2.555	.866	0.924	0.329–2.594	.880
24-h UFC 5th	0.743	0.261–2.120	.577	0.737	0.255–2.129	.572	0.744	0.254–2.180	.588
Age	1.086	1.068–1.104	<.001	1.064	1.043–1.087	<.001	1.063	1.039–1.087	<.001
Sex (female)	0.382	0.258–0.564	<.001	0.457	0.302–0.692	<.001	0.444	0.285–0.690	<.001
Previous smoker				1.306	0.709–2.405	.391	1.309	0.711–2.411	.387
Current smoker				2.768	1.532–5.001	.001	2.757	1.523–4.933	.001
Hypertension				2.515	1.467–4.310	.001	2.534	1.459–4.401	.001
Diabetes				1.307	0.692–2.470	.408	1.308	0.691–2.477	.409
Hyperlipidemia				1.564	1.020–2.398	.040	1.566	1.012–2.393	.044
CVD history				1.896	1.124–3.199	.017	1.871	1.101–3.180	.021
BMI							0.999	0.947–1.054	.979
Creatinine clearance							0.998	0.987–1.009	.726

**After exclusion of people on corticosteroid. medication. BMI=body mass index, 24-h UFC=twenty-four-hour urinary free cortisol, CVD=cardiovascular disease, HR=hazard ratio, 95% CI=95% confidence interval of the hazard ratio, p=p-value.*

The prediction of MACE by resting heart rate

After exclusion of persons using antihypertensive medication a population of 2823 people remained, in which 156 MACE occurred. Table 3 shows the results of the three Cox proportional hazard models assessing the association between quintiles of RHR and MACE. Compared to the lowest quintile, the risk for MACE was twice as high in the fourth and the fifth quintile while adjusting for sex and age. Only the fifth quintile of heart rate remained a significant predictor of MACE in the model adjusted for sex, age, hypertension, smoking status, hyperlipidemia, diabetes mellitus, and CVD history. In the final model, with additional adjustment for BMI and creatinine clearance, the fifth quintile remained a significant predictor of MACE. We repeated the analyses for the continuous variable of RHR (data not reported in table). Like the quintiles, the continuous variable of RHR was a highly significant predictor of MACE while adjusting for sex and age (HR=1.074, 95% CI=1.059-1.089, p<.001). After addition of hypertension, smoking status, hyperlipidemia, diabetes mellitus, and CVD history to the model, the continuous variable of RHR remained a significant predictor of MACE, but its effects were attenuated (HR=1.016, 95% CI=1.001-1.031, p=.036). In the final model, where also BMI and creatinine clearance were added the continuous variable of heart rate remained a significant predictor, increasing the risk for MACE with 16% per 10 extra heart beats per minute (HR=1.016, 95% CI=1.001-1.031, p=.036). We reran the same models after exclusion of people with a history of CVD. In fully adjusted models, the continuous variable of RHR (HR=1.016, 95% CI=1.001-1.031, p=.040) and the highest quintile (HR=2.073, 95% CI=1.184-3.629, p=.011) remained significant predictors of MACE in people without prior CVD.

Tabel 3. Association of MACE with resting heart rate in Multivariable Cox Regression Models*

	Model 1			Model 2			Model 3		
Variable	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
RHR 2nd	1.707	0.990–2.943	.054	1.643	0.953–2.833	.074	1.654	0.948–2.885	.076
RHR 3rd	1.026	0.541–1.948	.936	0.879	0.462–1.675	.695	0.895	0.467–1.716	.738
RHR 4th	2.026	1.180–3.476	.010	1.592	0.920–2.754	.096	1.635	0.938–2.850	.083
RHR 5th	2.626	1.569–4.397	<.001	1.942	1.151–3.276	.013	1.934	0.137–3.290	.015
Age	1,074	1.059–1.089	<.001	1.073	1.056–1.090	<.001	1.067	1.049–1.084	<.001
Sex (female)	0.275	0.193–0.391	<.001	0.295	0.205–0.424	<.001	0.260	0.147–0.386	<.001
Previous smoker				1.246	0.751–2.066	.395	1.277	0.763–2.138	.351
Current smoker				3.104	1.957–4.925	<.001	3.252	2.034–5.202	.001
Hypertension				1.337	0.940–1.900	.106	1.286	0.887–1.845	.172
Diabetes				0.943	0.384–2.312	.897	0.942	0.383–2.138	.897
Hyperlipidemia				2.010	1.454–2.788	<.001	1.987	1.434–2.753	<.001
CVD history				1.733	0.948–3.168	.074	1.704	0.930–3.121	.085
BMI							1.049	1.002–1.098	.040
Creatinine clearance							0.993	0.986–1.001	.074

**After exclusion of people who use antihypertensive medication. BMI=body mass index, RHR=resting heart rate, CVD=cardiovascular disease, HR=hazard ratio, 95% CI=95% confidence interval of the hazard ratio, p=p-value.*

DISCUSSION

In the current study we investigated whether 24-h UFC levels and RHR were independent predictors of major fatal and non-fatal adverse cardiovascular events. Higher RHR did indeed significantly increase the risk of MACE. Unlike RHR and traditional cardiovascular risk factors, 24-h UFC did not significantly contribute to the risk of MACE.

Limitations

There are several limitations that need to be considered when interpreting our results. Heart rate is susceptible to influences from the environment, and although it was measured in a very standardized way, with a 10-minute recording, 24-h holter registration could have increased accuracy. Moreover, as PREVEND is an observational study, it can never establish a causal link as residual confounding might still exist. We did for instance not adjust for physical activity levels while physical fitness is associated with both heart rate[24] and cardiovascular outcomes. A recent study demonstrated, however, that heart rate is an independent risk factor for mortality after adjustment for cardiorespiratory fitness as indexed by VO_2-max[13]. Another limitation of the current study is that the PREVEND study suffered from attrition and no-show at the scheduled follow-up visit at T2 at which 24-h UFC was measured. Individuals with missing data had in general more cardiovascular risk factors and experienced more events. As participants needed to be event free until the second survey to be included in the current study, our negative results might also in part be due to a survivor selection bias. Furthermore, the occurrence of death from other than cardiovascular causes might have precluded participants from experiencing MACE (competing risk phenomena). This is, however, not different from the other cohort studies, the INCHIANTI and the WHITEHALL II study, where missingness was also related to lower socioeconomic status and having more cardiovascular risk factors. Complete case analysis can yield biased estimates[25]. Unlike the two other studies, we used multiple imputation as a superior technique to mitigate the effects of missingness and to preserve power as is advised also for survival analysis[25,26]. The outcome MACE was known for every participant including those that had dropped out. Yet, our conclusion remained the same with both complete case analysis and analyses of the imputed sets. We can, however, never exclude the possibility that selection bias negatively influenced our results.

Strengths

The PREVEND study also has several strengths. It is a large population representative cohort in which both predictors and outcomes were well measured. MACE were assessed by using the Dutch national registry of hospital discharge diagnoses and the Dutch national bureau of statistics. Therefore, detailed information was available on both fatal and non-fatal events. Furthermore, medication use was substantiated with information from the database of pharmacy-dispensing data. Compared to the INCHIANTI study, the only other study which measured 24-h UFC, the PREVEND study has some important methodological strengths. Firstly, in our study, in the majority of cases, 24-h UFC was measured on two consecutive days thus giving a more reliable estimate of 24-h UFC levels, whereas in the INCHIANTI study only one day of 24-h urine was available. Secondly, we were able to verify completeness of the sample by measuring urinary creatinine excretion. We had to exclude 16% of the samples

based on this analysis. In the INCHIANTI study, compliance was assessed by self-report and therefore only 2.7% of samples was excluded. Thirdly, in the INCHIANTI study 24-h UFC was measured by Bayer's ADVIA-Centaur immunoassay system which has poor specificity due to cross-reactivity with cortisone (44%) and is sensitive to drug interference[27]. In PREVEND, 24-h UFC was measured by means of LC-MS/MS which is free of interferences from cortisol metabolites and conjugates, and also eliminates drug interferences[28].

Cortisol levels and cardiovascular outcomes

Three studies investigated the relationship between cortisol levels and cardiovascular mortality[11,12,29] Two found cortisol levels to be predictive cardiovascular mortality (a flatter slope of salivary cortisol levels over the day[12] in the WHITEHALL II, or higher levels of 24-h UFC [11] INCHIANT study respectively). Whereas one, the Vietnam Experience study, did not find an association between serum cortisol levels and cardiovascular mortality[29]. None of these studies took non-fatal events into account as we did. Yet, if higher cortisol levels really do increase the risk of CVD one would expect to also find an association with non-fatal events, which we did not. Our negative results cannot be explained by a lack of events. In the WHITEHALL II study and the CHIANTI study 32 and 41 fatal cardiovascular events took place respectively, whereas in PREVEND 121 MACE took place. In general 10 events per predictor variable are needed to get reliable regression estimates[30]. Lacking events does not just decrease power to detect a true effect, but also biases regression coefficients, and can also lead to an overestimation of the hazard[30]. The PREVEND study differs from WHITEHALL II and the INCHIANTI study on several other aspects. In terms of average age (52 years) PREVEND has a relatively young population compared to the aforementioned studies, where the average participant was older than 65 years. Furthermore, PREVEND is a general population based cohort, whereas participants in WHITEHALL II were white collar workers, and the participants of the INCHIANTI study were all retired. It might thus be that cortisol forms a risk for cardiovascular events only in an elderly population. We tested for this possibility by including and interaction term between 24-h UFC and age into our model, but found no statistical support for this hypothesis.

An explanation for our null findings might be that within-individual stability of cortisol levels are low. The few studies that assessed intra-individual stability of cortisol levels over longer periods of time showed perplexingly high intra-individual variability[31,32]. This would make it difficult to detect a true effect of 24-h UFC on MACE. Perhaps longer term indices of within-person levels of cortisol, such as cortisol measured in scalp hair[33], are more suitable to study the relationship between HPA-axis functioning and the occurrence of MACE.

Resting heart rate

In patients with CVD, resting higher RHR has consistently been found to predict cardiovascular mortality[16,18,34,35]. In the current study, we demonstrated in a relatively young and healthy population that RHR increases the risk of MACE while adjusting for various conventional risk factors. Our study adds to the accumulating epidemiological evidence from general population cohorts that RHR is a significant predictor of fatal and non-fatal cardiovascular outcomes in populations without preexisting CVD[13 15,36,37]. From the literature it is unclear whether the relationship between RHR and cardiovascular outcomes is linear. Some studies

have demonstrated a J-shaped relationship[38] or threshold effect[34], whereas other showed a clear linear relationship[16,39]. In patients with stroke, RHR has been demonstrated to increase the risk of myocardial infarction[35]. Several other studies failed to demonstrate a significant relationship between RHR and non-fatal cardiac events in patients with a history of CVD[16–18].

RHR seems to be a modifiable risk factor and not just a risk marker, as reduction of heart rate has proved to be protective against cardiovascular mortality For instance, in the BEAUTIFUL study, heart rate reduction with Ivabradine, a selective inhibitor of the I_f current, was shown to decrease the risk of cardiovascular death in patients with coronary artery disease and left ventricular dysfunction who had a RHR >70 beats per minute[39]. Likewise, in the SHIFT study, in patients with systolic heart failure, heart rate reduction with Ivabradine led to a decrease in cardiovascular deaths and hospitalizations[40]. It remains to be seen whether selective reduction of RHR could also be protective in other patient groups or in populations with elevated heart rate without established CVD.

In conclusion, RHR is a predictor of fatal and non-fatal major adverse cardiovascular events in the general population without pre-established CVD. This might have clinical implications, as the measurement of heart rate is a non-invasive low-cost procedure that could easily be used to detect people that are at heightened risk for a cardiovascular event. Finally, we did not find any evidence that higher urinary cortisol levels increase the risk of MACE.

REFERENCES

1. Kivimaki M, Nyberg ST, Batty GD, Fransson EI, Heikkila K, Alfredsson L, Bjorner JB, Borritz M, Burr H, Casini A, Clays E, De Bacquer D, Dragano N, Ferrie JE, Geuskens GA, Goldberg M, Hamer M, Hooftman WE, Houtman IL, Joensuu M, Jokela M, Kittel F, Knutsson A, Koskenvuo M, Koskinen A, Kouvonen A, Kumari M, Madsen IE, Marmot MG, Nielsen ML, Nordin M, Oksanen T, Pentti J, Rugulies R, Salo P, Siegrist J, Singh-Manoux A, Suominen SB, Vaananen A, Vahtera J, Virtanen M, Westerholm PJ, Westerlund H, Zins M, Steptoe A, Theorell T, Consortium I-W. Job strain as a risk factor for coronary heart disease: a collaborative meta-analysis of individual participant data. *Lancet*. 2012;380:1491–1497.
2. Steptoe A, Kivimaki M. Stress and cardiovascular disease. *Nat Rev*. 2012;9:360–370.
3. Whitworth JA, Kelly JJ, Brown MA, Williamson PM, Lawson JA. Glucocorticoids and hypertension in man. *Clin Exp Hypertens (New York, NY 1993)*. 1997;19:871–884.
4. Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JM. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension*. 1999;33:1364–1368.
5. Small GR, Hadoke PW, Sharif I, Dover AR, Armour D, Kenyon CJ, Gray GA, Walker BR. Preventing local regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase type 1 enhances angiogenesis. *Proc Natl Acad Sci U S A*. 2005;102:12165–12170.
6. Funder JW. RALES, EPHESUS and redox. *J Steroid Biochem Mol Biol*. 2005;93:121–125.
7. Tanaka N, Nozawa T, Yasumura Y, Futaki S, Hiramori K, Suga H. Heart-rate-proportional oxygen consumption for constant cardiac work in dog heart. *Jpn J Physiol*. 1990;40:503–21.
8. Bombardini T, Gemignani V, Bianchini E, Venneri L, Petersen C, Pasanisi E, Pratali L, Alonso-Rodriguez D, Pianelli M, Faita F, Giannoni M, Arpesella G, Picano E. Diastolic time - frequency relation in the stress echo lab: filling timing and flow at different heart rates. *Cardiovasc Ultrasound*. 2008;6:15.
9. Fox KM, Ferrari R. Heart rate: a forgotten link in coronary artery disease? *Nat Rev Cardiol*. 2011;8:369–79.
10. Heidland UE, Strauer BE. Left ventricular muscle mass and elevated heart rate are associated with coronary plaque disruption. *Circulation*. 2001;104:1477–82.
11. Vogelzangs N, Beekman AT, Milaneschi Y, Bandinelli S, Ferrucci L, Penninx BW. Urinary cortisol and six-year risk of all-cause and cardiovascular mortality. *J Clin Endocrinol Metab*. 2010;95:4959–4964.
12. Kumari M, Shipley M, Stafford M, Kivimaki M. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. *J Clin Endocrinol Metab*. 2011;96:1478–1485.
13. Jensen MT, Suadicani P, Hein HO, Gyntelberg F. Elevated resting heart rate, physical fitness and all-cause mortality: a 16-year follow-up in the Copenhagen Male Study. *Heart*. 2013;99:882–7.
14. Okamura T, Hayakawa T, Kadowaki T, Kita Y, Okayama A, Elliott P, Ueshima H. Resting heart rate and cause-specific death in a 16.5-year cohort study of the Japanese general population. *Am Heart J*. 2004;147:1024–1032.
15. Hsia J, Larson JC, Ockene JK, Sarto GE, Allison MA, Hendrix SL, Robinson JG, LaCroix AZ, Manson JE. Resting heart rate as a low tech predictor of coronary events in women: prospective cohort study. *BMJ*. 2009;338:b219.
16. Ho JE, Bittner V, Demicco DA, Breazna A, Deedwania PC, Waters DD. Usefulness of heart rate at rest as a predictor of mortality, hospitalization for heart failure, myocardial infarction, and stroke in patients with stable coronary heart disease (Data from the Treating to New Targets [TNT] trial). *Am J Cardiol*. 2010;105:905–11.
17. Legeai C, Jouven X, Tafflet M, Dartigues JF, Helmer C, Ritchie K, Amouyel P, Tzourio C, Ducimetière P, Empana JP. Resting heart rate, mortality and future coronary heart disease in the elderly: the 3C Study. *Eur J Cardiovasc Prev Rehabil*. 2011;18:488–97.
18. Bemelmans RHH, van der Graaf Y, Nathoe HM, Wassink AMJ, Vernooij JWP, Spiering W, Visseren FLJ. The risk of resting heart rate on vascular events and mortality in vascular patients. *Int J Cardiol*. 2013;168:1410–5.
19. Pinto-Sietsma SJ, Janssen WM, Hillege HL, Navis G, de ZD, de Jong PE. Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. *J Am Soc Nephrol*. 2000;11:1882–1888.

20. Knuiman JT, Hautvast JG, van der Heyden L, Geboers J, Joossens J V, Tornqvist H, Isaksson B, Pietinen P, Tuomilehto J, Poulsen L. A multi-centre study on completeness of urine collection in 11 European centres. I. Some problems with the use of creatinine and 4-aminobenzoic acid as markers of the completeness of collection. *Hum Nutr Clin Nutr*. 1986;40:229–37.
21. Murakami K, Sasaki S, Takahashi Y, Uenishi K, Watanabe T, Kohri T, Yamasaki M, Watanabe R, Baba K, Shibata K, Takahashi T, Hayabuchi H, Ohki K, Suzuki J. Sensitivity and specificity of published strategies using urinary creatinine to identify incomplete 24-h urine collection. *Nutrition*. 2008;24:16–22.
22. Visser ST, Schuiling-Veninga CCM, Bos JHJ, de Jong-van den Berg LTW, Postma MJ. The population-based prescription database IADB.nl: its development, usefulness in outcomes research and challenges. *Expert Rev Pharmacoecon Outcomes Res*. 2013;13:285–92.
23. Monster TBM, Janssen WMT, de Jong PE, de Jong-van den Berg LTW. Pharmacy data in epidemiological studies: an easy to obtain and reliable tool. *Pharmacoepidemiol Drug Saf*. 2002;11:379–84.
24. Sandvik L, Erikssen J, Thaulow E, Erikssen G, Mundal R, Rodahl K. Physical fitness as a predictor of mortality among healthy, middle-aged Norwegian men. N Engl J Med. 1993;328:533–537.
25. Marshall A, Altman DG, Holder RL. Comparison of imputation methods for handling missing covariate data when fitting a Cox proportional hazards model: a resampling study. *BMC Med Res Methodol*. 2010;10:112.
26. White IR, Royston P. Imputing missing covariate values for the Cox model. *Stat Med*. 2009;28:1982–98.
27. Gray G, Shakerdi L, Wallace AM. Poor specificity and recovery of urinary free cortisol as determined by the Bayer ADVIA Centaur extraction method. *Ann Clin Biochem*. 2003;40:563–5.
28. Taylor RL, Machacek D, Singh RJ. Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone. *Clin Chem*. 2002;48:1511–9.
29. Phillips AC, Carroll D, Gale CR, Lord JM, Arlt W, Batty GD. Cortisol, DHEA sulphate, their ratio, and all-cause and cause-specific mortality in the Vietnam Experience Study. *Eur J Endocrinol*. 2010;163:285–92.
30. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. *J Clin Epidemiol*. 1995;48:1503–10.
31. Schubert C, Geser W, Noisternig B, Fuchs D, Welzenbach N, Konig P, Schussler G, Ocana-Peinado FM, Lampe A. Stress system dynamics during “life as it is lived”: an integrative single-case study on a healthy woman. *PLoS One*. 2012;7:e29415.
32. Ross KM, Murphy MLM, Adam EK, Chen E, Miller GE. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. *Psychoneuroendocrinology*. 2014 Jan;39:184-93
33. Manenschijn L, Schaap L, van Schoor NM, van der Pas S, Peeters GMEE, Lips P, Koper JW, van Rossum EFC. High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. *J Clin Endocrinol Metab*. 2013;98:2078–83.
34. Diaz A, Bourassa MG, Guertin M-C, Tardif J-C. Long-term prognostic value of resting heart rate in patients with suspected or proven coronary artery disease. *Eur Heart J*. 2005;26:967–74.
35. Fox K, Bousser M-G, Amarenco P, Chamorro A, Fisher M, Ford I, Hennerici MG, Mattle HP, Rothwell PM. Heart rate is a prognostic risk factor for myocardial infarction: a post hoc analysis in the PERFORM (Prevention of cerebrovascular and cardiovascular Events of ischemic origin with teRutroban in patients with a history oF ischemic strOke or tRansient isc. *Int J Cardiol*. 2013;168:3500–5.
36. Shaper AG, Wannamethee G, Macfarlane PW, Walker M. Heart rate, ischaemic heart disease, and sudden cardiac death in middle-aged British men. *Br Heart J*. 1993;70:49–55.
37. Jensen MT, Marott JL, Allin KH, Nordestgaard BG, Jensen GB. Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. *Eur J Prev Cardiol*. 2012;19:102–8.
38. Kolloch R, Legler UF, Champion A, Cooper-Dehoff RM, Handberg E, Zhou Q, Pepine CJ. Impact of resting heart rate on outcomes in hypertensive patients with coronary artery disease: findings from the INternational VErapamil-SR/trandolapril STudy (INVEST). *Eur Heart J*. 2008;29:1327–34.

39. Fox K, Ford I, Steg PG, Tendera M, Robertson M, Ferrari R. Heart rate as a prognostic risk factor in patients with coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a subgroup analysis of a randomised controlled trial. *Lancet*. 2008;372:817–21.

40. Böhm M, Swedberg K, Komajda M, Borer JS, Ford I, Dubost-Brama A, Lerebours G, Tavazzi L. Heart rate as a risk factor in chronic heart failure (SHIFT): the association between heart rate and outcomes in a randomised placebo-controlled trial. *Lancet*. 2010;376:886–94.

SUPPLEMENT

Multiple imputation

The analyses of only complete cases may suffer more chance variation, and that under the missing at random assumption, multiple imputation should correct biases that may arise in complete cases analyses. We investigated whether the MAR assumption was tenable by testing if missingness was dependent on observed variables (including those on baseline) in our dataset. This was indeed the case. In order to preserve power and decrease bias in the estimates [1], we performed multiple imputation (MI) using Multivariate Imputation by Chained Equations (MICE) of any of the predictor variable with missing values. In addition, to all baseline and follow-up variables that predicted missingness, the MI model included all predictor variables that were in the final cox regression models, the outcome indicator D, and the Nelson-Aalen estimator as advised [2]. We imputed 100 datasets and graphically assessed if the MICE algorithm converged properly by plotting the means of the imputed variables against the iteration number and concluded that 10 iterations led to healthy convergence. The conclusions drawn from the cox regression models on the original data and the imputed data sets were in accordance with each other. As pooled estimates from imputed data are considered superior to those from complete case analysis, the estimates in the manuscipt are those of the imputed data. Estimates of the 100 imputed sets were pooled using Rubin's rules.

Percentage of missingness

Percent of missingness on the variables of interest for the T2 data (compared to the total population at T1) was as follows: history of cardiovascular disease (0.3%), RHR (17.2%), survival time (17.6%), use of antihypertensive medication (3.8%), use of glucocorticoid medication (0%), BMI (19,2%), smoking status (17,9%), hypertension (17,2%), hyperlipidemia (18,6%), diabetes mellitus (19,4%), cardiovascular events (MACE) (0%), 24-h UFC, after checking completeness by means of urinary creatinine output (31.9%).

Feasibility of the MAR assumption

People with missing values were significantly more likely to smoke 39.8 vs 32.9 (p=.001), had higher heart rate, 70 vs 68 beats per minute (p=.001), were less likely to do sports 67% vs 51% (p<.001), had a higher BMI 29 vs 26 (p<.001), higher fasting glucose levels 5.2 vs 4.9 (p<.001), were older 51 vs 58 years (p<.001), were more likely to be female 67% vs 49% (p<.001), had higher cholesterol 5.5 vs 5.4 mmol/L (p =.023), were more likely to have hypertension 42% vs 27% (p<.001), diabetes 10% vs 4%, hyperlipidemia 30% vs 22% (p<.001), to never drink alcohol 36% vs 22% (p<.001), and to experience MACE 10% vs 7% (p<.001). There were no significant differences in antihypertensive treatment 14% vs 17% (p=.074). As missingness depends on observed variables in the dataset. We conclude that it is feasible to use the information contained in the observed variables to impute the missing data.

REFERENCES

1. Marshall, A., Altman, D. G., & Holder, R. L. Comparison of imputation methods for handling missing covariate data when fitting a Cox proportional hazards model: a resampling study. *BMC Med Res Methodol.* 2010;10:112
2. White, I. R., & Royston, P. Imputing missing covariate values for the Cox model. *Stat Med. 2009;28:1982-98*